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Abstracts selected for a poster presentation

Abstract 1

HORMONE RECEPTOR STATUS IN PRIMARY POSTMEUOPAUSAL BREAST CANCERS. IS THERE AN ASSOCIATION WITH HORMONE REPLACEMENT THERAPY?

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1. Objectives

Some postmenopausal women with a malignant breast lump present while taking hormone replacement therapy (HRT). Data regarding the effect of HRT on oestrogen (ER) and progesterone receptor (PR) expression are inconclusive. We compared ER and PR expression in breast cancer tissue from HRT-users with those who never used it.

2. Methods

All consecutive postmenopausal women with an operable primary breast cancer ($n=67$) admitted in the St.-Jan's Hospital, Brussels in the year 2000, were included in a retrospective way. 26 women reported HRT use at the time of diagnosis whereas 41 breast cancer patients never used HRT. For each patient, we recorded age and body mass index (BMI) at the time of first treatment. Formalin-fixed tumour tissue from the primary breast cancer was immunohistochemically (IHC) examined using the monoclonal antibodies NCL-ER-6F11 for ER and NCL-PGR-312 for PR. The interpretation of the IHC-staining intensity is semiquantitative and subjective. We opted for the immunoreactive scoring system (IRS) of Remmele and Stegner [1].

3. Resu1ts

Both ER and PR were negative (IRS $\leqslant 2$) in 34.6% of the HRT-users whereas this was the case in 24.4% of the women never having used HRT. In those who expressed ER (IRS >2), the mean intensity of receptor staining was 7.8 in the HRT-users and 8.0 in the never-users. In those who expressed PR (IRS >2), the mean intensity of receptor staining was 6.3 in the HRT group and 6.5 in the never-users. Descriptive statistics are presented in the table. All the distributions were Normal (K-S test; $P>0.05$).

	N	Mean Age (S.D.)	Mean BMI (S.D.)	Mean ER (S.D.)	Mean PR (S.D.)
HRT+	26	65.1 (8.7)	25.9 (4.0)	4.9 (4.3)	2.9 (3.6)
ER+	16	65.7 (7.7)	25.8 (4.3)	7.8 (2.7)	
PR+	11	68.2 (7.1)	26.6 (4.8)		6.3 (3.0)
ER/PR−	9	62.4 (9.4)	26.5 (3.7)		
HRT−	41	75.0 (8.5)	26.7 (4.7)	6.1 (4.0)	3.6 (3.4)
ER+	31	74.1 (8.4)	26.9 (5.2)	8.0 (2.6)	
PR+	21	73.6 (7.4)	26.8 (6.0)		6.5 (2.4)
ER/PR−	10	77.7 (8.6)	26.2 (3.3)		

S.D., Standard Deviation.

HRT-users and non-users differed significantly for age (*t*-test; $P<0.001$), but not for BMI, mean ER and mean PR. In multivariate analyses, HRT-use (yes/no), age, and BMI appeared to be poor predictors of either ER intensity in ER expressors (multiple regression; $r^2=0.06$) or PR intensity in PR expressors ($r^2=0.121$). We performed a logistic regression analysis to measure the effect of HRT use on ER or PR qualitative expression ($\leqslant 2$ or >2), adjusted for age and BMI: HRT-users were less likely to express ER Odds Ratio (OR) = 0.43; 95% Confidence Interval (CI): 0.12–1.54) or PR (OR = 0.77; 95% CI: 0.24–2.47).

4. Conclusion

Our results suggest that HRT-users are more likely to have ER/PR-negative breast cancers compared with

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never-users, even considering that in our series HRT-users were younger and that age may play an important role in hormone receptor status. The observed association is not statistically significant, but this may be attributed to a lack of power (too few subjects). Nevertheless, this association should deserve further attention. Once the tumour expressed the hormone receptor (IRS > 2), comparing HRT-users with never-users, there was no difference in intensity of expression, neither for the ER nor for the PR.

References

1. Remmele, W, Stegner, H.E. ***Frauenarzt* 1987 28**, 41–43

Abstract 2

CHEMOGENE TREATMENT CONSISTING OF CYCLIN D1 ANTISENSE ODN, DOCETAXEL AND VINORELBINE ERADICATES CHEMORESISTANT HUMAN METASTATIC BREAST CARCINOMA OVEREXPRESSING ER, N-Ras AND HER-2/neu

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Approximately 50% of breast cancers contain overexpressed oestrogen receptors (ER) and 30% contain defective/overexpressed HER-2/neu. Oestrogen and HER-2/neu receptors may preferentially activate endogenous N-Ras protein in breast cancer cells. The cyclin D1 oncoprotein is found in abnormally high amounts in 50–60% of human breast tumours and it is associated with oestrogen receptor status and prognosis. Chemoresistant cancer genes *HER2/neu* and *N-Ras* can only send signals through the cyclin D1 protein. We obtain tumour cells from a chemoresistant patient with metastatic breast cancer (MBC). Immunohistochemistry (IHC) showed overexpression of HER-2/neu, N-Ras, cyclin D1 and ER. We aimed to eradicate these tumour cells by inactivating the HER-2/neu–N-Ras pathway via cyclin D1 (CD1) downregulation with a CD1 antisense-oligodeoxynucleotide (ODN) which targets the *CD1* mRNA blocking its signalling and allowing the induction of apoptosis after antimitotic treatment with vinorelbine and docetaxel. Post treatment, we observed a downregulation of cyclin D1 after treatment with the 18-mer phosphorothioated antisense oligodeoxynucleotide (ODN) with the unmethylated CG-dinucleotides (CpG motifs). This caused inactivation of the HER-2/neu and N-Ras pathways leading to their downregulation. In addition, IHC studies showed a downregulation of ER. Inactivation of HER-2/neu and NRas led to a circumvention of chemoresistance allowing vinorelbine and docetaxel to exert their antimitotic action eradicating the MBC cells via the induction of apoptosis programmed cell death (PCD) by activation of the caspase-3/CPP32 pathway. tRANSMISSION ELECTRON MICROSCOPY (TEM) exhibited morphological signs of the D2 apoptotic stage forming apoptotic bodies which were phagocytosed by adjacent tumour cells leading to a bystander killing effect (dimethylthiazoyl-2,5-diphenyltetrazolium bromide (MTT), and bromodeoxyuridine (BrdU) analysis of treated MBC cells showed a decrease in the metabolic activity and DNA synthesis, respectively, compared with untreated controls. In conclusion, by blocking cyclin D1, we completely inactivated the chemoresistant pathways of HER-2/neu and N-Ras leading to eradication of oestrogen-dependent metastatic breast carcinoma after treatment with vinorelbine and docetaxel.

Abstract 3

INDUCTION OF ADCC AND PCD WITH SUBSEQUENT BYSTANDER KILLING EFFECT IN INFILTRATING LOBULAR BREAST CARCINOMA CELLS (ILBC) AND LYMPHATIC/VASCULAR ENDOTHELIAL CELLS AFTER TREATMENT WITH rhu Mab KDR/FLK-1 (VEGFR-2) LINKED ONTO PEGYLATED LIPOSOMAL VINORELBINE TARTRATE (PLVT)

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The majority of ILBC when they become hypoxic secrete the vascular endothelial growth factor (VEGF) which induces endothelial cell proliferation. The VEGFR-2 (KDR/Flk-l) receptor transduces the effects of VEGF into endothelial cells. Anti-mouse VEGF receptor MAb produced by a hybridoma was humanised by inserting its murine KDR antigen-binding regions into the framework of a human immunoglobulin molecule. The recombinant DNA-derived humanised MAb binds to the extracellular domain of the VEGFR-2. Fab fragments of rhu anti-KDR mAb were covalently conjugated to polyethylene glycol-phycoerythrin (PEG-PE) linkers which were inserted into pH-sensitive liposomes composed of DOPE bilayers in which vinorelbine (VRL) was encapsulated. This complex is termed as pegylated liposomal vinorelbine tartrate (PLVT). Hypoxia induces VEGF in the acidic pericellular milieu of tumours where the liposomal bilayer structure is destabilised. Our aim is to target tumour growth inducing programmed cell death (PCD) in both activated endothelial cells and tumour cells enhancing TI of VRL by circumventing biological milieu interactions. We tested the amount of endothelial cell proliferation and apoptosis of HUVECs in the presence of PLVT. HUVECs were grown in medium from a patient

with ILBC for 8 h. Fluorescent activated cell sorting (FACS) analysis was used to determine PCD while formazan treatment assayed proliferation. Western blots (WB) were performed to assess the expression of VEGFR-2 and bcl-2 which promotes endothelial cell survival. Angiogenesis *in vivo* was examined using a Matrigel plug assay in nude mice. Treatment with PLVT was commenced a week after the Matrigel injection. We have also used the CAM assay to study the interaction of ILBC with the lymphatics *in vivo*. Inhibition of lymphangiogenesis was observed after treatment with PLVT. Flow cytometry, WB, immunohistochemistry (IHC), BrdU, MTT, TdT-mediated dUTP biotin nick-end labelling (TUNEL) and TEM analysis was performed in the ILBC and endothelial cells before and after treatment with PLVT. Anti-KDR mAb by blocking the binding epitopes on KDR receptor inhibited VEGF-stimulated phosphorylation of this receptor in HUVEC. Subsequently, antiKDR mAb inhibited the mitogenic stimulation of HUVEC by VEGF. PLVT treatment reduced up to 80% of vascularisation observed. We have developed an *in vivo* xenotransplant model where the effect of PLVT in inhibiting the growth of ILBC was seen in immunocompromised mice. All animals were sacrificed on day 14. For both endothelial and tumour cells, flow cytometry showed arrest at the G2/M phase and interruption of the mitochondria transmembrane potential with the release of cytochrome-c. Fluorometric caspase protease assay kits showed activation of the caspase-9 protease (initiator) after association of Apaf-1 with cytochrome-c forming an Apaf-1/cyt-c complex. Caspase-9 directly cleaves and activates caspase-3 (effector). TEM results showed that caspase-3 cleaved the cytoarchitecture leading to blebbing and the nuclear lamina leading to nuclear breakdown, while it activated DNAase causing a breakdown of DNA and subsequent laddering. This resulted in D2 apoptotic signs with the formation of apoptotic bodies that were phagocytosed by adjacent tumour cells leading to a bystander killing effect. MTT and BrdU analysis showed a large decrease in the metabolic activity and DNA synthesis of the treated tumour cells compared with controls. TEM showed an antimicrotubule action on both endothelial and tumour cells. There was a downregulation of VEGFR-2 leading to an inhibition of angiogenesis and lymphangiogenesis of the endothelial cells according to BrdU proliferation studies. bcl-2 expression of was downregulated due to phosphorylation by VRL. A cytotoxic activity of human K-cells containing immunoglobulin Fc receptors was observed indicating ADCC as a result of the anti-KDR antibody binding to the KDR receptor on endothelial cells. Finally, PLVT treatment induced ADCC and significantly reduced tumour cell proliferation due to PCD in both ILBC and endothelial cells of the lymphatics and vasculature which might inhibit metastatic spread in ILBC. Thus, this synergistic therapeutic strategy may be of future benefit against infiltrating lobular breast carcinoma.

Abstract 4

IS WEIGHT OF THE BREAST ASSOCIATED WITH HORMONE RECEPTOR EXPRESSION IN POSTMENOPAUSAL WOMEN WITH INVASIVE BREAST CANCER?

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1. Objective

In postmenopausal women, oestrogens are produced from the aromatisation of C19 steroids in stromal adipose cells of peripheral and breast fat. Enzymes in breast cancer tissue modulate intratumoral levels of oestrone, oestradiol and oestrone sulphate. We examined how the endogenous oestrogenic environment is associated with steroid hormone receptor expression. We hypothesised that the oestrogen driven progesterone receptor (PR) is more likely to be present in breast cancers from obese patients, in those with large size breasts and large tumours. We also expected an ER + breast cancer to be of larger size in an oestrogen-rich environment compared with an oestrogen-poor situation.

2. Methods

Retrospectively, we examined the effect of weight of mastectomy and tumour size on ER and PR-expression in 139 postmenopausal women with an invasive breast cancer. Age and tumour differentiation were also taken into account. In a subgroup of 82 of these 139 women, ER and PR expression were also examined in relation to the same set of variables, plus the woman's body mass index (BMI). Hormone steroid receptors were analysed by immunohistochemistry using the H-score and $\geqslant 30$ was chosen as a positive value (range: 0–300). We used standard statistical procedures (Pearson correlation, linear regression, logistic regression) when distributions proved to be normal, and non-parametric tests otherwise (Spearman correlation, Chi-square test).

3. Results

In the 139 women, as expected, hormone receptor expression (positive or not) was strongly associated with

tumour differentiation (chi-square test; $P > 0.001$ for both receptors). Levels of Oestrogen Receptor (ER) and PR were strongly correlated (Spearman rho = 0.5; $P < 0.001$). We observed no significant association between other variables. Noteworthy, breast weight and tumour diameter were not associated with ER (< 30, $\geqslant 30$) (Mann–Witney test; $P = 0.930$ and 0.617) or PR expression ($P = 0.452$ and 0.810).

In the subgroup of 82 women, breast-weight was strongly correlated with BMI (Pearson correlation = 0.8; $P < 0.01$). We performed a logistic regression analysis to examine the relationships between age, BMI, breast weight, tumour diameter and tumour differentiation, and qualitative ER expression (65 ER+ vs 17 ER−). Tumour differentiation was only significantly associated with ER expression (Wald test; P: 0.002). We also performed a linear regression analysis in the 65 ER-positive women in order to model ER expression by taking into account the same set of covariates. The model did not fit at all (r square = 0.046. all P-values > 0.05), providing no evidence of any significant relationship between the independent variables considered and ER expression. When we tried to model PR expression by logistic regression analysis, it appeared that tumour differentiation ($P < 0.01$), BMI ($P = 0.051$), but not breast weight, and tumour diameter ($P = 0.036$) were positively associated with PR expression. However, after selection of the 50 PR-positive women, linear regression analysis failed to identify any variable associated with the magnitude of PR expression.

4. Conclusion

Using the now generally accepted method to measure steroid hormone expression in invasive breast cancers, immunohistochemistry, in our series of postmenopausal women, we failed to demonstrate any clinically relevant and statistically significant adjusted effect of age, BMI, breast-weight or tumour diameter on ER expression. Tumour differentiation was the only variable associated with ER positivity. Yet, we observed a significant adjusted effect of BMI, tumour diameter and tumour differentiation on PR status, albeit these variables did not appear to influence the level of PR expression in women who scored positive.

Abstract 5

OVARIAN CYST FORMATION IN TAMOXIFEN-TREATED BREAST CANCER PATIENTS: A CLINICAL DILEMMA?

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Tamoxifen is widely used as long-term primary adjuvant and/or palliative therapy for patients with breast cancer. Currently, tamoxifen is also investigated for the prevention of breast cancer in high-risk women. Only recently, ovarian cyst formation has been reported as a possible side-effect on the female genital tract. In this scientific poster, three consecutive tamoxifen-treated (40 mg) breast cancer patients with ovarian cysts are presented:

Patient A was a 43 years old woman with metastatic breast cancer, referred with a request for palliative laparoscopic ovarian ablation. In spite of chemotherapeutic treatment she was still menstruating regularly. Transvaginal ultrasound revealed multilocular ovarian cysts (> 7 cm) on both sides. (E2 level: 2.5 nmol/l). Both ovaries were removed. Histopathology: benign follicular cysts.

Patient B was a 57 years old postmenopausal woman with metastatic breast cancer, referred on behalf of a pelvic mass. Transvaginal ultrasound revealed a large (> 20 cm) ovarian cyst with solid particles. (E2 level: 0.1 nmol/l; CA-125 level 81 kU/l). By laparotomy, a cystic ovarian tumour was resected. Histopathology: benign mucinous cystadenoma.

Patient C was a 38 years old premenopausal woman, referred with a request for palliative laparoscopic ovarian ablation because of metastatic breast cancer. Preoperative transvaginal ultrasound reveals a multilocular cyst (> 5.7 cm) of the right ovary, ascites is present. (CA-125 level 81 kU/l). Both ovaries were removed laparoscopically. Histopathology: normal ovarian morphology with corpus luteum.

It is concluded that, as indications for tamoxifen use are increasing, ovarian cyst formation may pose more and more therapeutical problems for clinicians. For instance, tamoxifen-induced ovarian cysts in premenopausal women can lead to paradoxical high E2 levels. Ovarian cyst formation might increase the risk of ovarian cancer in these women, who already bear a higher chance on the basis of their potential genetic predisposition (*BRCA* l and *BRCA* 2).

In postmenopausal tamoxifen-treated women, ovarian cyst formation appears controversial; the question arises of pure coincidence or a causative relationship? Secondly, the preoperative use of CA-125 in patients with metastatic breast cancer may not be warranted as being sensitive for ovarian malignancy.

Abstract 6

DISTINCT FUNCTIONAL DIFFERENCES OF HUMAN PROGESTERONE RECEPTORS A AND B ON GENE EXPRESSION AND GROWTH REGULATION IN TWO ENDOMETRIAL CARCINOMA CELL-LINES

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Progesterone plays an important role in controlling the proliferation and differentiation of the human endometrium. The human progesterone receptor (hPR), via which progesterone mediates its effects, exists as two isoforms: hPR-A and hPR-B. To study the functional differences of two isoforms in human endometrial cancer, a well differentiated hPR-negative Ishikawa cell-line was stably transfected with either hPR-A or hPR-B. Two new endometrial carcinoma cell-lines were created; one expressing hPR-A (PRA-14) and one expressing hPR-B (PRB-59). Differences in the expression of genes targeted by the two isoforms were studied using a cDNA expression array technique. A different set of genes appeared to be progesterone regulated in the PRA-14 cells compared with the PRB-59 cells. Sixteen genes were progesterone regulated in the PRA-14 cells and four genes in the PRB-59 cells. None of the genes were regulated by both hPR-A and hPR-B. Cell growth experiments revealed a growth inhibitory response to progestins (MPA and R5020) in the PRB-59 cells, but not in the PRA-14 cells. In conclusion, a new model to study functional differences between the two hPR isoforms in human endometrial carcinoma cells has been established. This model revealed distinctive differences in target genes between the two hPR isoforms. Moreover, the antiproliferative actions of progesterone on human endometrial cancer cells could only be observed in the PRB-59 cells and not in the PRA-14 cells.

Abstract 7

8-PRENYLNARINGENIN, THE PHYTOOESTROGEN IN HOPS AND BEER, MAY INFLUENCE THE INCIDENCE OF BREAST CANCER

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Breast cancer is a major health concern in menopausal women. Conflicting data have been published on the influence of consuming alcohol-containing drinks on the risk of breast cancer. Data largely focus on the alcohol content of different beverages. Oestrogenic effects have been reported on occupational exposure of females working in the hop processing industry. 8-Prenylnaringenin (8-PN) was recently discovered as the principal phytooestrogen in hops and beer. The relationship between oestrogens and breast cancer has been inferred from observations that early menarche and late menopause and hormonal replacement therapy after menopause increase the incidence of breast cancer. We investigated the effect of 8-PN on aggregation, growth and invasion in the human MCF-7 breast cancer cell line family.

MCF-7/6 cell showed a poor tendency to aggregate in slow aggregation assays, in accordance with previous results. Treatment with 8-PN at 10 and 1 μM concentrations had a beneficial effect on the aggregation, as was evident from the formation of larger and more compact aggregates. A significant growth stimulatory effect was observed in cultures treated with different concentrations of 8-PN. 8-PN had no effect on invasion in embryonic chick heart. 8-PN had similar effects as 17 beta-oestradiol: it promotes cell-cell adhesion and growth without an effect on invasion. We can only speculate on the clinical relevance of our data with 8-PN. In beers concentrations of 8-PN up to 3×10^{-7} M are present. Further studies are required to demonstrate if beer consumption can result in mammary tissue concentrations of 8-PN that are comparable to the active concentrations of our studies *in vitro*. The consumption of beer may interfere with the incidence of breast cancer in patients enrolled in studies on hormonal substitution therapy.

Abstract 8

OESTROGEN AND PROGESTERONE AND ANDROGEN RECEPTORS IN OVARIAN CANCER: CORRELATION WITH UROKINASE-TYPE PLASMINOGEN ACTIVATOR CONCENTRATION

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The aim of the present work was the evaluation of the expression for oestrogen, progesterone and androgen receptors in tumour cytosols Oestrogen Receptor (ER), Progesterone Receptor (PR) and Androgen Receptor (AR) of ovarian cancer based on the level of urokinase type plasminogen activator (uPA).

Enzyme-linked immunosorbent assay (ELISA) kits developed by Beraard's group (Nijmegen. The Netherlands) were used for the detection of uPA. ER PR and AR were determined by the standard radioligand dextran-coated charcoal (DCC) assay. 71 primary ovarian cancer patients aged between 23 and 76 years (median: 54 years; mean 52.3 ± 2.1) were investigated. ER was found in 32% of the tumours, PR—in 70%, and AR—in 38%. The concentration of uPA in the tumor cytosols consisted of 0.01–6.52 ng/mg protein. The uPA

concentration in the tumour cytosols for ER-positive and ER-negative tumours was similar (0.85 and 0.88 ng/mg protein, respectively. The uPA concentration for PR- and AR-negative tumour cytosols was 1.16 and 1.32 ng/mg protein, respectively, and in PR- and AR-positive tumour cytosols was 0.68 and 0.41 ng/mg protein, respectively. The negative correlation between the level of PR and uPA was statistically significant ($R = -0.35$; $P = 0.02$). As an increased uPA level is associated with a metastatic cancer and a poor prognosis of ovarian cancer, our results may suggest a positive prognosis for cases of ovarian cancer with PR- and AR-positive tumours.

Abstract 9

OESTROGEN RECEPTOR ALPHA PROTEIN IS MORE PREVALENT IN THE MYOMETRIUM OF BLACK SOUTH AFRICAN WOMEN SUFFERING FROM UTERINE LEIOMYOMA COMPARED WITH CAUCASIAN WOMEN

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1. Objective

Uterine leiomyomas develop in women of reproductive age and regress after menopause, suggesting that they grow in a steroid hormone-dependent fashion. Furthermore it is widely accepted that uterine leiomyomas are more common in African-Americans compared with Caucasian women.

2. Materials and methods

We analysed DNA ploidy of uterine leiomyomas and also investigated the presence of the progesterone receptor (PR) protein, the oestrogen receptor α (ERα) protein and the cell proliferation marker Ki-67 in uterine myometrium and leiomyomas of Caucasian and black South African women.

3. Results

A total of 43 premenopausal women were included. None were on hormonal treatment and all were in the secretory phase of their menstrual cycle. Women were asked for cofactors in the pathophysiology of uterine leiomyomas such as cigarette smoking and body mass index. ERα stained more in the myometrial nuclei from black South African women than in Caucasian women ($P = 0.0075$). Multiple linear regression analysis identified race as the only indicator of ERα positivity ($P = 0.0043$). The difference in ERα positivity between the races was not observed for uterine leiomyomas ($P = 0.7$). Furthermore, PR and Ki-67 were not different between the races, either in the myometrium, or in uterine leiomyomas. DNA ploidy was comparable in uterine leiomyomas from both races.

4. Conclusion

These results suggest that the ERα is more prevalent in the myometrium of black South African women suffering from uterine leiomyomas compared with Caucasian women. We hypothesise that this difference in human myometrial biochemistry contributes to the higher incidence of uterine leiomyomas in African women.

Abstract 10

CUMULATIVE DOSAGE EFFECT OF CYTOGENETICALLY DEREGULATED *RAD51L1* and *HMGIC* IN PSEUDO-MEIGS' SYNDROME

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1. Background

Uterine leiomyoma presenting with ascites and pleural fluid is called pseudo-Meigs' syndrome. Interestingly, it is unclear whether 'normal' uterine leiomyomas and uterine leiomyomas causing pseudo-Meigs' syndrome are cytogenetically related or whether functionally different primary pathogenetic triggers are responsible for the clear differences in the observed tumour phenotype. In this report, we investigated the possible involvement of the *RAD51L1* and *HMGIC* genes in initiation and or progression of a huge uterine leiomyoma presenting as pseudo-Meigs' syndrome.

2. Results

The detailed cytogenetic and fluorescent *in situ* hybridisation (FISH) analysis revealed the presence of two subclones with a complex karyotype 46,XX,der(2)t(2;12)(q31;q21),der(12)t(2;12)(q31;q15).ish der(12)t(2;12)(LL12NC01-142H1−;LL12NC01-27E12-), ish del(12)(ql 5q15) (LL100-2NCO1-142H1-;LL12NCO1–27E12-), ins (14;12)(q23;ql5q21).ish ins(14;12)(LL12NC0I-142H1 +;LL12NC01–27E12 + x2, RAD51Li +)[20]/46,idem, del(14)(q21q23).ish(RAD51 LI-)[6], indicating intragenic *HMGIC* rearrangement and loss of one of the *RAD51L1* alleles in a derivative subclone with chromosome 14 deletion. Surprisingly, rapid amplification of CDNA ends (RACE) and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis on the tumour cells did not reveal abnormal *HMGIC* or *RAD51L1* transcripts. Additionally, the cellular subclone with intrachromosomal 14q21-23/*RAD51L1* deletion showed an *in vitro* growth advantage over the subclone without the deletion.

3. Conclusion

We postulate that the cumulative dosage effect of simultaneous *HMGIC* as well as *RAD51L1* deregulation plays a role in pseudo-Meigs' syndrome.

Abstract 11

PRELIMINARY EVALUATION OF TIBOLONE EFFICACY IN PREVENTING MENOPAUSAL SYMPTOMS IN BREAST CANCER PATIENTS

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1. Aim

Evaluation of tibolone in preventing heavy menopausal symptoms in patients after breast cancer treatment.

2. Material and methods

14 patients were treated in our Cancer Center between 1998 and 2001 demonstrating strong menopausal symptoms significantly worsening their quality of life. In 12 cases, ductal breast cancer was diagnosed and in 2 other cases medullar type cancer. Oestrogen and progesterone receptors content was determined in all cases. Kupperman index was used to evaluate the quality of life. Tibolone in a dose of 2.5 mg/day was introduced after 3 months of ineffective treatment with alternative methods. All patients were under oncological follow-up every 3 months. Average time of observation was 16 months.

3. Results

In all cases menopausal symptoms decrease significantly during tibolone treatment. After 6 months of tibolone treatment, the mean value of the Kupperman index was 12 points and after 1 year 8 points. Two patients gave up further treatment because of anxiety against recurrence of cancer. During the follow-up period no signs of recurrence were found.

4. Conclusion

Tibolone (2.5 mg/day) significantly reduces the menopausal symptoms improving quality of life. Tibolone may be a safe and effective agent used as hormone replacement therapy (HRT) in selected groups of breast cancer patients.

Abstract 12

HORMONE RECEPTOR EXPRESSION IN FLEMISH PATIENTS WITH *IN SITU* AND INVASIVE PRIMARY BREAST CANCER

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In Flanders, 34 centres report their new gynae oncology cases to the registry of the 'Flemish Gynae Oncology Group'.

This report includes data from 1998–2000 on steroid hormone receptors from 306 'in situ' and 3138 'invasive' breast cancers and answers two questions. The first question wonders whether steroid hormone receptors in breast cancers are differently expressed between the two menopausal periods. The second question finds out whether an oestrogen dependent breast cancer (as determined from the oestrogen (ER) and progesterone receptor (PR) status) is less likely to spread to the axillary lymph nodes (ALN).

We defined 'oestrogen dependent' as tumours with the following steroid hormone expression: ER positive/PR positive, ER positive/PR negative, ER negative/PR positive. Only the ER negative/PR negative cases are listed as oestrogen independent. We obtained the steroid hormone receptor status from 'in situ' breast cancers in 45.1% (138/306) and from 'invasive' breast cancers in 88.1% (2764/3138) of the cases.

For the 'in situ' breast cancers we found out that 79.1% (38/48) of the lesions were oestrogen dependent in premenopausals whereas this figure was 84,4% (76/90) for postmenopausals. For the 'invasive' group (Table 1), in premenopausal women 77% (636/826) were oestrogen-dependent whereas this figure was 82.7% (1598/1938) in postmenopausals. The difference between both menopausal groups for invasive breast cancer was statistically significant at a p value of 0.015.

Table 1
ER/PR status per menopausal period

ER/PR status	Premenopausal	Postmenopausal
ER pos/PR pos	497 (60.2%)	1218 (63%)
ER pos/PR neg	86 (10.4%)	328 (17%)
ER neg/PR pos	53 (6.4%)	52 (2.7%)
ER neg/PR neg	190 (23%)	340 (17.3%)

Table 2
ER/PR and ALN status: Premenopausals

ER/PR status	ALN status	Number
ER pos/PR pos	Negative	282
	Positive	189
ER pos/PR neg	Negative	41
	Positive	25
ER neg/PR pos	Negative	35
	Positive	16
ER neg/PR neg	Negative	100
	Positive	73

Table 3
ER/PR and ALN status: Postmenopausals

ER/PR status	ALN status	Number
ER pos/PR pos	Negative	677
	Positive	422
ER pos/PR neg	Negative	169
	Positive	129
ER neg/PR pos	Negative	29
	Positive	13
ER neg/PR neg	Negative	168
	Positive	125

In premenopausal women with an invasive breast cancer without ALN involvement, 78.1% (358/458) of the breast lesions were oestrogen dependent whereas this was in 75.9% (230/303) in those with ALN involvement. In postmenopausal women this figure was 83.8% in those where the cancer didn't spread to the ALN and 81.8% in case of lymph node involvement (Tables 2 and 3).

In conclusion: Breast cancer and carcinoma in situ are often oestrogen dependent and this in both menopausal periods; women with ALN involvement of their breast cancer are not more likely to have an oestrogen independent tumour compared with those without ALN involvement.

Abstract 13

EXPRESSION OF PROLACTIN RECEPTOR mRNA IN BREAST CANCERS PRE- AND POST TAMOXIFEN TREATMENT

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1. Aim

To determine whether treatment with tamoxifen results in the downregulation of the prolactin receptor in breast tumours, so providing the basis for a test to predict responsiveness to anti-oestrogen therapy.

2. Methods

28 post-menopausal women were treated with tamoxifen (20 mg daily). Biopsy specimens were obtained with ultra-sound guidance immediately before the initiation of treatment, and again after treatment for seven days. RNA was extracted from the biopsies, and prolactin receptor (*PRLR*) mRNA determined by reverse-transcription polymerase chain reaction (RT-PCR). Results were expressed relative to 18S ribosomal RNA, which was also measured by RT-PCR. Oestrogen receptor, progesterone receptor, pS2, c-Erb-B2 and Bcl-2 were determined by immunocytochemistry on a second sample taken before the initiation of tamoxifen treatment.

3. Results

There was a significant decrease in *PRLR* expression ($P = 0.005$), with a particularly marked decrease ($> 60\%$) in a sub-group of ten patients. The decrease in *PRLR* mRNA was greater in those cancers which did not express Cerb2, compared with the Cerb2-positive cancers ($P = 0.014$).

4. Conclusions

Treatment with tamoxifen for one week results in a highly significant down-regulation of PRLR expression which, in over one third of breast tumours, is profound. This response may reflect, in part, the mechanisms by which tamoxifen exerts its clinical effect.

Abstract 14

IMMUNOHISTOCHEMICAL INVESTIGATION OF OESTROGEN AND PROGESTERONE RECEPTOR EXPRESSION IN PRIMARY BREAST CANCER AND THEIR AXILLARY LYMPH NODE METASTASES

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1. Objectives

Axillary and distant metastases from invasive breast cancer may represent a clonal outgrowth with a different hormone receptor status than the primary tumour and this may explain why metastatic oestrogen receptor (ER)-positive breast cancer may not respond to anti-oestrogens. We examined the ER and progesterone receptor (PR) expression in the primary breast cancer and its metastatic axillary lymph nodes.

2. Methods

We randomly selected 21 patients with node-positive invasive ductal adenocarcinoma of the breast for which tissue blocks were available from both the primary cancer and the axillary lymph node metastases. Single samples ($n = 84$) of formalin-fixed tumour tissue from the primary breast cancer and the involved lymph node were immunohistochemically (IHC) examined using the monoclonal antibodies NCL-ER-6F11 for ER and NCL-PGR-312 for PR. The interpretation of the IHC reactivity is semiquantitative and subjective. We opted for the immuno-reactive scoring system (IRS) of Remmele and Stegner, 1986 ($IRS = SI \times PP$ *(SI is the intensity of the nuclear marquage from 0 (negative) to 3 (strong)—PP is the percentage of positive cells from 0 (no immunomarquage) to 4 (more than 80% positive cells)*). The IRS scoring system gives us values from 0 to 12. Three independent readers gave a staining score from 0 to 12 for each tissue sample with a calculated mean being the sum of each divided by three.

3. Results

Seven primary tumours were ER negative (0/12). In 5 women, the involved nodes were also ER-negative (0/12) but in two, ER expression in the axillary lymph node was discordant: ER was present in one with a score of 2/12 and in the other with a score of 6/12. Fourteen patients had ER-positive breast cancer (2–12/12). All had ER expression in tumour tissue from the axillary node. Staining intensity was identical in 4, less in 5 but more intense in 5 others.

Eleven primary tumours were PR-negative (0/12). Three women had PR-expression in the involved axillary node with low expression intensities of 3/12, 3/12 and 2/12.

Ten women had PR-positive breast cancers (2-9/12). One such patient didn't express PR in the metastatic lymph node, 6 stained positive for PR with an identical intensity and 3 had a less intense staining in the metastatic axillary node.

4. Conclusion

In most women with invasive breast cancer, hormone receptor expression in the primary tumour is concordant with hormone expression in the metastatic axillary lymph node. Staining intensity may be different (more or less) between the primary lesion and tumour tissue in the axillary node. Two and 3 of the 21 women expressed the ER or PR, respectively, in the axillary lymph node whereas the primary tumour was hormone receptor-negative. Only one patient had hormone receptor negative tumour tissue in a lymph node while the primary tumour was positive; this was only for the PR and not for the ER.

There is no need for systematic measurement of hormone receptor expression of tumour tissue in metastatic axillary lymph nodes from breast cancer. Whether anti-oestrogens are useful in the odd patient with hormone receptor-positive disease in the axillary lymph node and receptor-negative disease in the primary tumour is unknown, but may partially explain why some ER-negative breast cancer patients benefit from tamoxifen.